

Protocol for CRISPR Fluorescence Test

To obtain the complex of sgRNA and Fluc-dCas9 fusion protein, sample solution is prepared by adding 3 μ L 1000nM sgRNA (100nM final) and 2 μ L 0.25uM fusion protein into 8.5 uL of 1X HEPES buffer (20mM HEPES, 150mM KCl, pH 7.5), and incubated at 25°C for 10 mins.

Add 3 μ L 30nM target (3 nM final) to mixture of two protein-sgRNA complexes and incubated at 37°C for 30 minutes. Pipet samples (30uL per well) into a 96-well black-welled plate (warmed to 37°C). Add 100uL of Luciferase Assay Reagent (Promega) to a sample and promptly measure the luminescence (1000ms) in Microplate Reader (Thermo).